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MEASUREMENT OF THE CATALYTIC POWER OF CATALASE

L. G. M. BAAS BECKING AND H. C. HAMPTON

I. INTRODUCTION

In this paper we propose to describe a new method and a new principle of determining the strength of catalase action. We do not endeavor to sum up all the literature existing, the excellent works of C. Oppenheimer[1] and H. Euler[2] having made this unnecessary. Of the literature which has appeared since 1909 we have taken only such work into account as showed an immediate connection with ours. Therefore we merely mention the elaborate work of G. B. Reed.[3]

The present problem originated from a proposed research on autofermentation in *Cannabis sativa* L. The first enzym to be dealt with was catalase. There were many difficulties to be overcome before we could start the work. The easiest way is to take commercial peroxide and let it act on crude plant juice, determining either the oxygen discharged by the method described by H. H. Bunzel[4] or the peroxide decomposed by means of titration with permanganate of potassium—the latter the method of nearly all other authors. With both methods we made determinations, but were very soon convinced that we were not ascertaining the actual strength of the enzym. One aim of the present paper, therefore, is to attempt to prove the inadequacy of the existing methods.

The value of a new method depends entirely on three factors: (1) The purity of the enzym; (2) the purity of the peroxide; and (3) the way of determining the action of the enzym on the peroxide. We took care only of the third factor in our experiments, though for the sake of completeness we mention all three. The first factor may vary according to the different aims of the research.

For studies in kinetics (R. O. Hertzog[5]), the enzym must be free from crystalloid, from peroxidase, and from impurities. For physiological research the methods of precipitation, be it with strong alcohol, inorganic salts or lead acetate, or by dialysis and centrifuging, enfeeble the enzym to

[1] Die Fermente, Zehnte Aufl Leipzig, 1909.

[2] Grundlagen und Ergebnisse der Pflanzenchemie, 2ter Teil. 1909.

[3] Bot. Gaz. 1915–1918.

[4] Journ. Biol. Chem. 20. 1914.

[5] Zeitschr. Physik. Chem. 41. 1904; Oppenheimer, Part II.

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a marked degree and are therefore to be avoided as much as possible. All authors, however, agree that, although the influence of accompanying salts may not be very great, damage by acid is considerable (G. Senter[6]). Therefore the physiologist must take care to neutralize the juice. A neutralization with Na_2CO_3 worked in our experiments very satisfactorily. In experiments in which the same juice is used for several days, a few drops of toluol must be added as a preservative.

The impurity of commercial peroxide should not be overlooked. It contains acid (even the perhydrol of Merck) which must first be neutralized. Also, most of these peroxides contain a certain amount of acetanilide ($\pm 1/15$ percent). G. Senter has proved that aniline is poisonous to catalase. Therefore it is better to work with purified peroxide (methods by G. Bredig[7]). However, we shall try to show that, working with our method, the results are not greatly influenced by the impurities of the enzym or of the peroxide. We worked in these preliminary experiments with commercial 10-volume peroxide and freshly prepared, mostly undiluted, plant juice, neutralized during the grinding. We shall try the same method later on with purified chemicals and enzym.

The third factor, that of the method of determining the quantity of oxygen liberated or of peroxide decomposed, is the most important one. There are three methods for the quantitative measurement of catalase.

1. The method of Palladin (cited by A. Kasanski[8]) consists in measuring the height of the foam developed during the reaction. There is, however, no sufficient ratio between the intensity of the phenomenon and catalase activity. The purer the enzym, the smaller the volume of foam, etc.

2. Titration with potassium permanganate. This is the method used by nearly all authors, but we have considerable doubt that it will serve its purpose. In the first place, organic compounds of different kinds oxidize permanganate. Therefore the press juice of itself, has a certain oxidizing power. This power is difficult to measure, not only because the end point of the reaction (a permanent red color) is difficult to observe, but also because the fluid is often so much colored that color reactions cannot be measured. These facts are stated by P. Waentig and A. Steche[9] and by W. Issajew.[10] The latter does not mention his method, so that the value of his results could not be judged. Permanganate shows this uncertain end point also with all kinds of organic salts (citrates, malates, tartrates) as we were able to prove. Therefore it is not surprising that even excellent scientists like Bach sometimes made considerable errors with the titration method (A. M. Clover[11]). Notwithstanding our working with the usual

[6] Zeitschr. Physik. Chem. **51.** 1905.
[7] Zeitschr. Physik. Chem. **31.** 1899.
[8] Biochem. Zeitschr. **39.** 1912.
[9] Zeitschr. Physik. Chem. **72, 76, 79, 83.** 1911–1915.
[10] Zeitschr. Physik. Chem. **42.** 1905.
[11] Amer. Chem. Journ. **29.** 1904.

precaution, we distrust the value of the numbers we obtained with the permanganate method.

3. We come now to the manometrical methods. These lack the advantage of the titration method in working under normal pressure. Furthermore, the fluid may reach the unfavorable condition of over-saturation with gas (P. Waentig and A. Steche, *l.c.*). Of the authors whose work has been done by this method we name: C. H. Appleman,[12] W. W. Bonns,[13] H. H. Bunzel (*l.c.*), W. E. Burge,[14] C. Foa,[15] W. B. Magath,[16] W. Zaleski and Anna Rosenberg.[17] The chief objections to this method are: (1) The pressure becomes higher during the reaction. We feel justified in disregarding the effect of over-pressure as our check experiments have shown this to be negligible. (2) The solution contains a great part of the oxygen. This is true only for narrow vessels, in which the surface is small in proportion to the volume of air. Even in the apparatus of H. H. Bunzel (*l.c.*) we feel that there is danger of the fluid becoming oversaturated. Bunzel tried to avoid this danger by shaking. But R. O. Hertzog (*l.c.*) cites a list of cases in which enzyms are destroyed by shaking. For instance, P. Waentig and A. Steche (*l.c.*) proved the destructive action of shaking on catalase.

It seemed clear to us, therefore, that if we chose the lesser of two evils, namely the manometrical method, we should take a container with a very broad bottom and a shallow layer of fluid. Experiments have shown us that the effect of shaking on the exchange of gas in such a column is minimal. In the short time of the catalase reaction the enzym is not injured by shaking. In more prolonged experiments (with oxidases for instance) it may be. There is another advantage in experimenting with a shallow layer of fluid since R. O. Hertzog (*l.c.*) proved that the catalase reaction is subject to the laws of diffusion, which is the most complete in thin layers.

II.

One more important criticism of nearly all methods of enzym determination is possible. To detect the fault we must start at the very beginning, at the definition of the word *enzym*. An enzym is a substance that *changes the velocity* of a reaction. Peroxide of hydrogen will decompose spontaneously but slowly. It will oxidize a certain amount in one month. Catalase changes the reaction time from one month to one minute. The only method theoretically justified would therefore be to determine the the time in which a reaction is completed under the influence of an enzym. That time is the measure of the enzym action.[18] If the reaction is monomo-

[12] Bot. Gaz. **50**. 1910.

[13] Ann. Mo. Bot. Garden **5**. 1918.

[14] Amer. Journ. Physiol. **44**. 1917.

[15] Biochem. Zeitschr. **11**. 1908.

[16] Journ. Biol. Chem. **24**. 1918.

[17] Biochem. Zeitschr. **33**. 1911.

[18] On the assumption that the reaction time *without* enzym is *very much* greater than that time *with* enzym.

lecular, and follows the law of mass action, the well-known formula of van't Hoff will apply:

$$\frac{dx}{dt} = k(a - x)$$

in which
a = available amount,
x = decomposed amount,
t = time,
k = reaction velocity.

Integration of this form $k = \frac{1}{0.4343t}\log\frac{a}{a-x}$ enables us to find the reaction velocity from a single determination. Concerning catalase a great variety of opinions exists in regard to the constancy of the reaction velocity. If the reaction velocity were proved to be practically constant, we should find, if x approaches its maximum value (let us say $\frac{999}{1000}a$):

$$k = \frac{3}{0.4343t}$$

or, k will be inversely proportional to t. In this case only would one be justified in measuring the so-called reaction-velocity, taking this as a comparative number for the "real" reaction-velocity, *i.e.*, $\frac{1}{\text{reaction time}}$. Excepting Bredig, who first called attention to this fact, F. A. F. C. Went[19] is the only author, so far as we know, who has tried to determine directly the time in which a reaction took place. He studied starch hydrolysis by the enzym of *Aspergillus niger*. His numbers are interpolated but still show marked properties.

In all cases, more or less scattered determinations (see, for example, figures in W. M. Bayliss[20] on glycerol-glucoside) must furnish the basis for the calculations. In our special case of catalase action, an *autographic method* which marks the time in which the reaction is ended offers a solution of this difficulty. Furthermore, this gives us opportunity to collect a far greater number of figures. As we learned after we had worked out the apparatus, the idea of an autographic record was not new.

C. Foa (*l.c.*) used a Mosso-plethysmograph and a revolving drum with soot paper to determine the action of different phenols on oxidase. He published his graphs without using them for calculation. A. Schultze (cited by Foa), used a self-recording manometer for measuring the CO_2 output in yeast activity. M. Antropoff studied autographically the periodical decomposition of peroxide by mercury. (Stephane Leduc[21] has explained his results in a peculiar way.)

[19] Verh. Kön. Akad. Wet. Amsterdam **27**. 1918.

[20] General physiology, 2nd edition. London, 1917.

[21] Théorie physico-chimique de la vie. Paris, 1910.

III.

We shall start now with the description of our own experiments.

We used a rather large reaction vessel connected with a manometer by a ground joint. The vessel was closed by a ground stopper. Joint and stopper were fastened on the bottle with strong rubber bands. On the ground stopper was sealed a small vessel with two holes. The small vessel contained the peroxide, while the larger held the enzym. The idea was borrowed from Haldane's well known apparatus for blood-gas determination. By turning the vessel in a plane perpendicular to the paper, the peroxide flowed from the smaller vessel into the larger one. In this plane the vessel could also be shaken. If the fluid contained catalase, oxygen would be liberated. This would increase the pressure in the vessel and the mercury column in the manometer would rise.

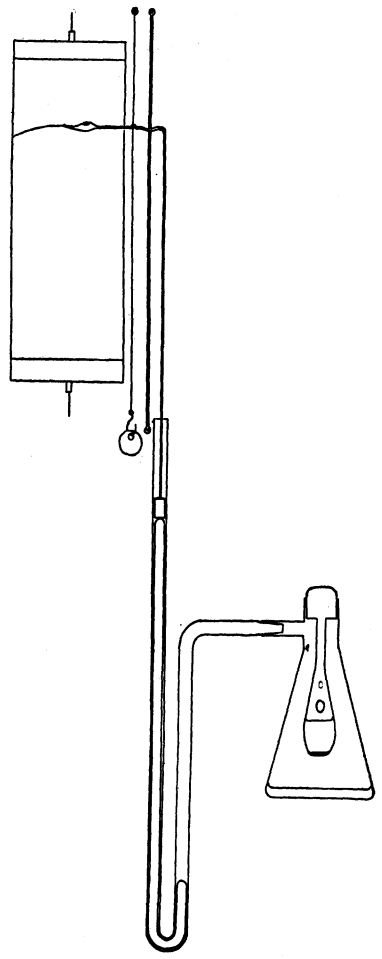

FIG. 1. Self-recording manometer with modified Haldane apparatus.

The autographic writer was simple to make. We had at least four methods from which to make our choice: (1) Transfer of the movement by levers; (2) transfer of the movement by air (Marey, Buisson); (3) direct record of mercury level by sensitive paper; (4) direct transfer.

The second method would not be the most efficient in our case, for, like the first method, it would record the results either enlarged or reduced. In the case of the third method we could not use coordinate paper. Therefore, we transcribed the pressure by means of a wooden float which carried a thin glass rod on which a glass pen was sealed at right angles to the rod. This we kept in position by a glass slide-bar and a weighted hair. This simple arrangement made it possible to register differences of $\frac{1}{20}$ mm. in the mercury level. The friction had an effect of $\frac{1}{10}$ mm. We therefore always calculated the records in millimeters. The efficient speed for the rotating drum was in our case one revolution in eight minutes for a diameter of 10 cm. The pen went over the distance of 1 mm. in $\frac{13}{8}$ seconds. The speed should be slower in the case of purer enzyms in which the action is feeble. The

flask stood inside the thermostat and could be shaken from the outside by means of a handle.

We obtained in normal cases a curve of the shape shown in figure 2.

At A (fig. 2) the peroxide is in contact with the enzym; at B the reaction begins; at C the reaction is completed. The distance A–B existed always

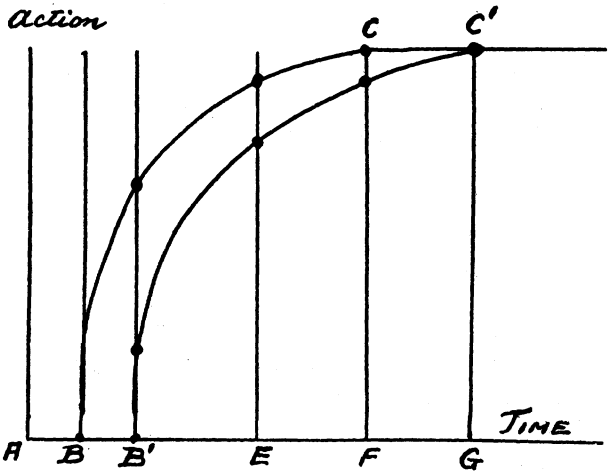

FIG. 2. For explanation see text.

and was not caused alone by the transportation time of the pressure. The distance A–B became greater when we used smaller quantities of enzym. This fact, namely, that oxygen is not immediately discharged, is mentioned only by P. Waentig and A. Steche (*l.c.*) With the use of the non-autographical methods, and especially with that of the titration method, this fact nearly always escapes observation. We saw the latency time (A–B) manifested, when the titration method was used, only in dilute enzym solutions. The small bubbles of oxygen were formed sometimes one minute after the beginning of the reaction. *It is clear, therefore, that the titration method not only is unfit to give us the end point of the reaction; it is unfit also to give us the moment of the beginning.*

The differing length of A–B with different enzym dilutions gives us a hint that the decomposition is caused by two successive reactions (A. Bach[22]), as follows:

1. $2H_2O_2 + 2A{=}O \rightarrow 2A\begin{matrix}O-OH\\OH\end{matrix}$. This unstable compound decomposes:

2. $2A\begin{matrix}O-OH\\OH\end{matrix} \rightarrow 2A\begin{matrix}O\\|\\O\end{matrix} + 2H_2O$ This peroxide is decomposed:

3. $2A\begin{matrix}O\\|\\O\end{matrix} \rightarrow 2A{=}O + O_2$ Summing up, we obtain

4. $2H_2O_2 \rightarrow 2H_2O + O_2$

[22] Chem. Ber. 36–42. 1904–1908.

This reaction (4) would be true when $A-B = 0$ seconds.

This is the reaction given by most authors. G. Bredig (*l.c.*), however, doubted the value of this equation. He found the reaction velocity constant. Therefore, he argues, the reaction must be monomolecular, and follow the scheme:

$$H_2O_2 \rightarrow H_2O + O$$

But the oxygen liberated is not atomic oxygen; it is not ionized, but molecular. T. H. Kastle and A. S. Loevenhart[23] defend on this ground the validity of the bimolecular equation. We are not able to follow their criticism of Bredig's work, and we will only remark that in the case in which $A-B>0$ the whole controversy seems to be solved. In fact, the first two reactions in the scheme of Bach are monomolecular.

IV.

We will compare now the action of two different quantities of enzym. The line A–B′–C′ (fig. 2) gives the action of the smaller quantity. What method must we follow to find out the ratio of their strengths?

1. *Reaction velocity.* The more accurate investigations deal with reaction velocity. We will show that the enzym is destroyed during the reaction (see below). Therefore the reaction velocity diminishes (sometimes very slightly) as nearly all authors have shown. (Issajew, *l.c.*, however, finds a constancy to the third decimal.) *The reaction velocity is therefore a misleading test for the strength of an enzym.*

2. *Amount of peroxide decomposed.* This method, though much used, has very little value, as figure 2 will demonstrate. A determination of the ratio in strength between A–B–C and A–B′–C′ would give:

At B′, 10:5;
E, 10:7;
F, 10:9;
G, 10:10.

Still we find in the literature on the subject, expressions like this: "There is three times as much catalase in the body wall of *Ascaris suum* as in the leg muscles of *Rana pipiens*" (Magath, *l.c.*).

3. A better method is the direct measurement of the reaction time (see above, II). This is possible only with a self-recording apparatus.

We prepared our materials by the method thus described. The tops of female hemp plants were ground in a meat-grinder with a small amount of powdered Na_2CO_3. The ground substance was then squeezed in a fruit press. The turbid fluid obtained is very stable and still strongly active after the lapse of fourteen days. The determinations all took place at 20° C. The peroxide was the usual commercial 10-volume H_2O_2, which

[23] Amer. Chem. Journ. **29**. 1903.

contains acetanilide enough to damage the enzym during the reaction (see below). We expect to repeat the experiments under standard conditions and ask the reader therefore to consider this paper as a preliminary account.

The time in which the reaction on 2 cc. peroxide was completed was in one case:

For 4 cc. extract, 15 mm. (15 $\times \frac{13}{8}$ sec.);
for 3 cc. extract, 21 mm. (21 $\times \frac{13}{8}$ sec.);
for 2 cc. extract, 29.5 mm. (29.5 $\times \frac{13}{8}$ sec.);
for 1 cc. extract, 59 mm. (59 $\times \frac{13}{8}$ sec.);
for $\frac{1}{2}$ cc. extract, 118 mm. (118 $\times \frac{13}{8}$ sec.).

We can state that the reaction time is inversely proportional to the amount of enzym, E (amount of enzym) $\times$ T (reaction time in units of $\frac{13}{8}$ seconds) thus being constant.

Again:

For 4 cc. extract, $E \times T = 60$;
for 3 cc. extract, $E \times T = 63$;
for 2 cc. extract, $E \times T = 59$;
for 1 cc. extract, $E \times T = 59$;
for $\frac{1}{2}$ cc. extract, $E \times T = 59$.

In another case we obtained these results:

For 4 cc. extract, $E \times T = 24$;
for 3 cc. extract, $E \times T = 27$;
for 2 cc. extract, $E \times T = 26$;
for 1 cc. extract, $E \times T = 25$;
for $\frac{1}{2}$ cc. extract, $E \times T = 24.5$.

These numbers strikingly show the value of the autographical method.

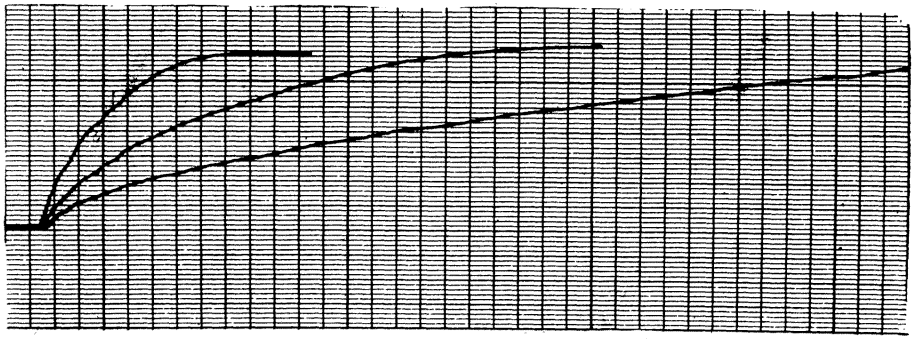

FIG. 3. Autogram. Effects of different quantities of enzym.

Figure 3 gives still another proof for 3 curves, taken with 4, 2, and 1 cc. of extract respectively, and 4 cc. 10-vol. H_2O_2. Different amounts of

peroxide change the time in the same manner. Figure 4 will illustrate this.

4 cc. of extract react with:

	Time in "mm."	$\frac{T}{\text{peroxide}}$
4 cc. 10-vol. peroxide	45	11.3 $\frac{(45)}{4}$
3 cc. 10-vol. peroxide	34	11.3 $\frac{(34)}{3}$
2 cc. 10-vol. peroxide	22	11. $\frac{(22)}{2}$
1 cc. 10-vol. peroxide	10	10 $\frac{(10)}{1}$

The times are proportional to the amount of peroxide.

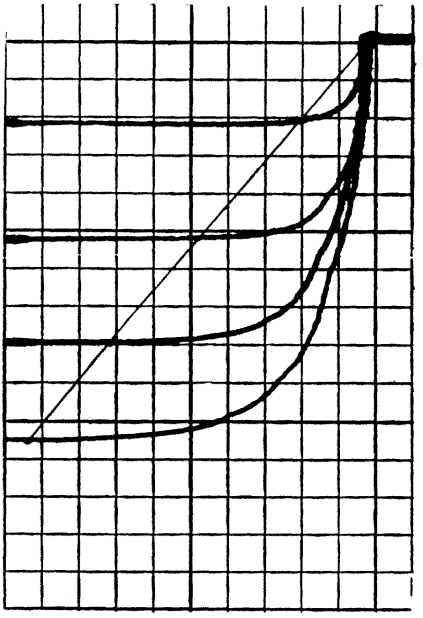

FIG. 4. Autogram. Effects of different quantities of peroxide.

To compare these results with the reaction velocities, we used the following method: We imagined the assymptote extended to the ordinate axis. Then the quantity $(a - x)$ of the formula

$$\frac{dx}{dt} = c\,(a - x)$$

will be the distance from a given point of the curve to the assymptote. We call it D. We can write for a point of the curve P:

$$\text{tg}\alpha_P = K_P D_P, \text{ or } K_P = \frac{\text{tg}\alpha_P}{D_P},$$

α_P being the angle between the tangent at the point P and the time axis.

We had only to measure α_P and D_P and we could read immediately on the slide rule the resulting K_P. We averaged a great number of K's taken from different curves. We also calculated the probable error. It proved to affect the units only. This must be taken into account.

Extract in cc.	Peroxide in cc.	Number of Experiments	Reaction Velocity	$\frac{\text{Peroxide} \times K}{\text{Enzym}}$
$\frac{1}{2}$	2	4	41×10^{-4}	164×10^{-4}
1	2	7	110×10^{-4}	220×10^{-4}
2	2	11	257×10^{-4}	257×10^{-4}
2	4	4	109×10^{-4}	218×10^{-4}
3	2	4	396×10^{-4}	264×10^{-4}
4	2	1	535×10^{-4}	263×10^{-4}
4	4	4	261×10^{-4}	261×10^{-4}
1	4	4	47×10^{-4}	188×10^{-4}

Considering the irregularity of the curves due to the poor clockwork and the possible differences in strength of the enzym solutions, $\frac{PK}{E}$ is a fairly approximately constant number. But it would take 39 determinations of this sort to prove what one determination of reaction time gave us, namely, that the reaction follows the law of mass action.

We tried to compare the curves obtained with mathematically constructed logarithmic lines. (Kapteyn used a similar method for Gaussian curves). We constructed several lines

$$t = c \log \frac{a}{a - x}$$

for c varying from 0.1 to 2 and a = 2 cm. We found in one case:

Calculated from Curve	Amount extract	C × E
0.15	4 cc.	0.6
0.2	3 cc.	0.6
0.3	2 cc.	0.6
0.6	1 cc.	0.6
1.2	0.5 cc.	0.6

Perhaps this method will be found to be the most practical and accurate.

The line A−B in which, according to our idea, the first part of the reaction must take place, becomes long enough to be measured in very feeble enzym concentrations only. Figure 5 shows curves run with 3 cc. peroxide and 4, 3, 2, 1, 0.5, 0.2, 0.1, and 0.05 cc. extract respectively, all diluted to 4 cc. fluid. The reaction started at the thick vertical line. The latency time caused by the apparatus (fig. 5) seems to be $2 \times \frac{13}{8}$ seconds. So we had to subtract 2 from the length A–B.

Latency time × *amount of enzym* seems to be more or less constant. But to draw conclusions from these facts seems premature.

Cc. extract	A-B	A-B Calculated for E × (A-B) = 1.5	E × (A-B)
4	Not measurable	0.38	—
			—
3		0.5	—
			—
2		0.75	—
			—
1		1.5	—
			—
0.5	1*	3	
0.2	7	7.5	1.4
0.1	15	15	1.5
0.05	32	30	1.6

* Out of place.

V.

There is still one assumption which we have not proved. How do we know that the reaction is finished at the point C of our curve (fig. 2), where

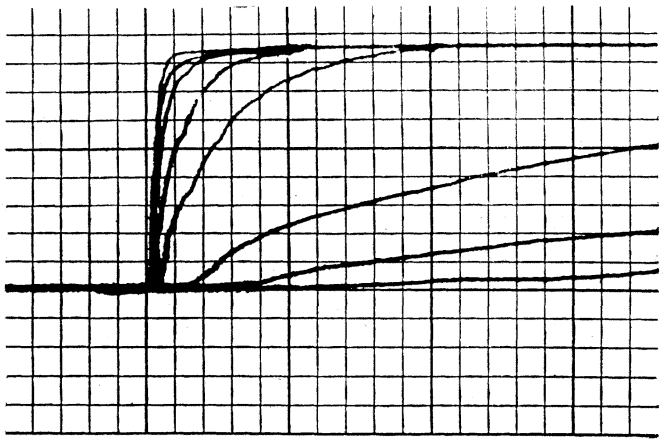

FIG. 5. Autogram showing latency times.

the curve becomes assymptote to the time axis? To determine this we have only to calibrate our flask. If the volume of the container to the level of the mercury is V_1, the volume of the peroxide V_H, and that of the extract V_E, the remaining volume before the beginning of the reaction will be $V_1 - (V_H - V_E)$. After the liberation of oxygen the mercury is forced down a cm., the volume of 1 cm. to be V_c cc., so the volume after the reaction will be

$$V_1 = (V_H + V_E) + aV_c.$$

If the temperature is constant during the experiment, we can use the simple formula of Boyle. If the pressure before the experiment be H cm. mercury, it will be (H + 2a) after the reaction. We get for the volume after the

reaction:

$$V_2 = \left\{ \frac{V_1 - (V_H + V_E) + aV_c}{H} \right\} (H + 2a)$$

or the oxygen produced

$$V_2 - \{V_1 - (V_H + V_E)\} = \frac{a}{H}[2\{V_1 - (V_H + V_E)\} + V_c(H + 2a)].$$

In one case we found:

$V_1 = 272.19$
$V_H = 2$
$V_E = 2$
$a = 2.3$
$H = 76$
$V_c = 0.29$

Oxygen discharged:

$$\frac{2.3}{76}[2 \times 268.19 + 0.29 \times 80.6] = 17.2 \text{ cc.}$$

Now the original peroxide was supposed to be 10-volume. We checked the experiment by titration at the same temperature and found 8.7-volume. This signifies that 2 cc. peroxide would yield 17.4 cc. oxygen.

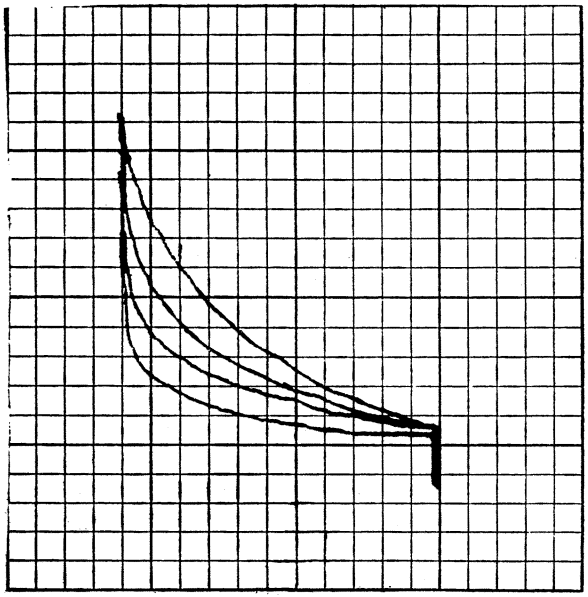

FIG. 6. Autogram showing the influence of successive doses of peroxide.

Thus we see that the autographic method can be used to determine the strength of a peroxide solution.

We can make this clear with an experiment done with 7.5-volume peroxide and 4 cc. extract. The autographic record showed:

Amount Peroxide in cc.	Final Pressure in cm.	cc. Oxygen Calculated From Titration	cc. Oxygen Calculated From Formula
1	1.2	7.5	8.2
2	2.2	15.	16.2
3	3.3	22.5	23.9
4	4.2	30.	28.2
5	5.6	37.5	37.3
6	6.8	45.	45.
7	8.	52.5	52.5
8	9.1	60.	59.6

Successive doses of 4 cc. peroxide on the same 4 cc. extract had the results shown in figure 6. There are two explanations possible. Either (1) the dilution of the solution affects the strength of the enzym, or (2) the enzym is "poisoned" by the peroxide.

We now made a determination of the influence of dilution by means of the titration method. From the results of this experiment we may conclude that the action of catalase does not vary with its dilution or with the quantity of the peroxide, but only with the absolute quantity of the enzym itself.

The results were:

Constitution of the Enzym Solution	Peroxide	Percent Peroxide Decomposed after 1 Minute
1 cc. extract, 0 H_2O	2 cc.	34.6%
1 cc. extract, 3 H_2O	2 cc.	29.8%
1 cc. extract, 8 H_2O	2 cc.	33.3%
1 cc. extract, 15 H_2O	2 cc.	30.9%
1 cc. extract, 24 H_2O	3 cc.	30.3%
1 cc. extract, 35 H_2O	2 cc.	31.8%
1 cc. extract, 48 H_2O	2 cc.	32.9%
1 cc. extract, 63 H_2O	4 cc.	26.9%

So only the second assumption is valid, the enzym is destroyed by the peroxide.

The reaction times of the successive amounts were:

	Time	Strength $= \frac{1}{\text{time}}$
1st dose	52 (× 1 5/8 sec.)	100
2d dose	58	89
3d dose	65	80
4th dose	75	69

± 10 percent of the enzym is destroyed during every successive reaction. This decrease in the reaction velocity supports the unproved assumptions of Bredig (*l.c.*).

The influence of alkali is very marked. Enzym solutions neutralized with Na_2CO_3 hold their power for days. Even neutralized hemp powder that had been dried for two weeks showed marked activity. There is a strong possibility that the alkali works as a "peptisator" on the enzym. Many peptisators are known in colloid chemistry, alkali acting very strongly

on albuminoids (Graham). The protein character of catalase is probable (Waentig and Steche, *l.c.*). The assumption of an α and a β catalase, proposed originally by O. Loew[24] would in that case be superfluous (compare E. Pozzi-Escot[25]) and Appleman (*l.c.*). The activity of the catalase declines very slowly on filtering, especially if the solution has been previously neutralized. In the latter case the activity decreased only 8 percent.

Unneutralized juices lose their catalytic power very soon. We found, for instance, in one case:

	Standing	Reaction Time	Strength
2 cc. extract neutralized	5′	46 units	100
2 cc. extract unneutralized	5′	85 units	54
2 cc. extract unneutralized	10′	151 units	31
2 cc. extract unneutralized	120′		0

There is evidence that this reaction follows also a logarithmic line.

Attempts to prepare the enzym in pure condition have failed. Unlike peroxidase, catalase adheres with a great tenacity to the alcohol precipitate.

We have refrained in the foregoing from discussing the physiological questions suggested by or even suggesting our work, for such a research can start only after the methods are worked out satisfactorily.

Summary

1. A review is given of the literature concerning the question. Difficulties and inaccuracies in several methods are pointed out.

2. According to the definition of an enzym, the reaction time is the only valid index of its strength. This strength can best be measured by an autographical method.

3. An autographical method is given. The method shows the evidence of two successive reactions.

4. The enzym is more or less injured or destroyed during the reaction. In most reactions the time is too short to influence markedly the logarithmic curve.

5. The method given is adapted to determine the strength of a peroxide solution.

6. There is evidence that the two different catalases are different degrees of peptisation of the same substance.

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Department of Botany,
Leland Stanford Junior University

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